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(FILE 'HOME' ENTERED AT 10:44:14 ON 28 JAN 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA'  
ENTERED AT

10:44:36 ON 28 JAN 2003

L1 10512953 S PEPTIDE OR POLYPEPTIDE OR OLIGOPEPTIDE OR PROTEIN  
OR RECEPTOR

L2 13965 S (FACTOR VIIA) OR (FACTOR VIIAI) OR (FFR-FACTOR VIIA) OR  
(GLUC)

L3 1457073 S (L1 OR L2) (P) (PURIF? OR ISOLAT?)

L4 30144 S ANION (W) EXCHANGE (W) (CHROMATOGRAPHY OR  
COLUMN)

L5 8956 S L3 (P) L4

L6 5826 S ORGANIC MODIFIER

L7 890678 S ALKANOL OR ALKYNOL OR ALKENOL OR UREA OR  
GUANIDINE OR (ALKNAO)

L8 0 S L5 (P) L6

L9 527 S L5 (P) L7

L10 0 S L9 (P) IMPURIT?

L11 103 S L9 (P) ELUT?

L12 37 DUPLICATE REMOVE L11 (66 DUPLICATES REMOVED)

L13 0 S L9 (P) (NEGATIVE CHARGE)

=> log y

| Type | L # | Hits | Search Text  | DBs   | Time Stamp                               | Comments             | Err or Def ro ini rs |
|------|-----|------|--|---|--|----------------------|----------------------|
| 1    | BRS | L1   | 41219<br>4<br>(factor adj VII) or<br>(factor adj VIIa) or<br>(FFR-factor adj VIIa)<br>or (glucagon-like adj<br>peptide-1) or<br>(glucagon-like adj<br>peptide-2) | peptide or<br>polypeptide or<br>oligopeptide or<br>protein or receptor<br>or vira or glucagon<br>or high orinsulin or<br>(factor adj VII) or<br>(factor adj VIIa) or<br>(FFR-factor adj VIIa)<br>or (glucagon-like adj<br>peptide-1) or<br>(glucagon-like adj<br>peptide-2) | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:43 | 0                    |
| 2    | BRS | L2   | 10404<br>69  | purif\$7 or isolat\$3   | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:44 | 0                    |
| 3    | BRS | L3   | 10506<br>2   | same 2  | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:44 | 0                    |
| 4    | BRS | L4   | 5161   | anion adj exchange<br>adj (chromatography<br>or column)   | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:45 | 0                    |
| 5    | BRS | L5   | 1643   | 3 same 4  | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:45 | 0                    |
| 6    | BRS | L6   | 374  | organic adj modifier  | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:46 | 0                    |
| 7    | BRS | L7   | 1  | C1-6-alkanol  | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:47 | 0                    |

| Type | L # | Hits | Search Text  | DBs                                      | Time Stamp                               | Comments             | Err or Err Def ro ini rs |
|------|-----|------|--|--|--|----------------------|--------------------------|
| 8    | BRS | L8   | alkanol or alkynol<br>or alkenol or urea or<br>guanidine or<br>(alkanoic adj acid)<br>or polyalcohol | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:49                     |                      | 0                        |
| 9    | BRS | L9   | 85   | 5 same (6 or 8)                          | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:49 | 0                        |
| 10   | BRS | L10  | 28079negative adj charge   | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:50                     |                      | 0                        |
| 11   | BRS | L11  | 1  | 9 same 10                                | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:52 | 0                        |
| 12   | BRS | L12  | 4  | 9 same impurity                          | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:56 | 0                        |
| 13   | BRS | L13  | 45875<br>9   | industrial                               | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 09:56 | 0                        |
| 14   | BRS | L14  | 2  | 9 same 13                                | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 10:01 | 0                        |
| 15   | BRS | L15  | 2  | stabv adj arne.in.                       | USPAT; US-PGPUB;<br>EPO; JPO;<br>DERWENT | 2003/01/2<br>8 10:01 | 0                        |

LE 'HOME' ENTERED AT 10:44:14 ON 28 JAN 2003

|  |            |         |
|--|------------|---------|
| file medline caplus biosis embase scisearch agricola | SINCE FILE | TOTAL   |
| OST IN U.S. DOLLARS                                  | ENTRY      | SESSION |
| ULL ESTIMATED COST                                   | 0.21       | 0.21    |

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LE 'CAPLUS' ENTERED AT 10:44:36 ON 28 JAN 2003  
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LE 'AGRICOLA' ENTERED AT 10:44:36 ON 28 JAN 2003

s peptide or polypeptide or oligopeptide or protein or receptor or vira or hgh or insulin or (f  
1 FILES SEARCHED...  
3 FILES SEARCHED...  
4 FILES SEARCHED...  
10512953 PEPTIDE OR POLYPEPTIDE OR OLIGOPEPTIDE OR PROTEIN OR RECEPTOR  
OR VIRA OR HGH OR INSULIN OR (FACTOR VII)  
s (factor VIIa) or (factor VIIai) or (FFR-factor VIIa) or (glucagon-like peptide-1) or (glucago  
4 FILES SEARCHED...  
13965 (FACTOR VIIA) OR (FACTOR VIIAI) OR (FFR-FACTOR VIIA) OR (GLUCAGO  
N-LIKE PEPTIDE-1) OR (GLUCAGON-LIKE PEPTIDE-2)

s 11 or 12  
1 FILES SEARCHED...  
3 FILES SEARCHED...  
4 FILES SEARCHED...  
COMMAND INTERRUPTED

this message appears repeatedly, please notify the Help Desk.  
Enter "HELP STN" for information on contacting the nearest STN Help  
desk by telephone or via SEND in the STNMAIL file.

s 11 or 12  
1 FILES SEARCHED...  
3 FILES SEARCHED...  
4 FILES SEARCHED...  
COMMAND INTERRUPTED  
this message appears repeatedly, please notify the Help Desk.  
Enter "HELP STN" for information on contacting the nearest STN Help  
desk by telephone or via SEND in the STNMAIL file.

s (11 or 12) (p) (purif? or isolat?)  
3 FILES SEARCHED...  
1457073 (L1 OR L2) (P) (PURIF? OR ISOLAT?)  
s anion (w) exchange (w) (chromatography or column)  
30144 ANION (W) EXCHANGE (W) (CHROMATOGRAPHY OR COLUMN)

s 13 (p) 14  
8956 L3 (P) L4

s organic modifier  
5826 ORGANIC MODIFIER

s alkanol or alkynol or alkenol or urea or guanidine or (alknaoic acid) or glycol or polyalcoho  
890678 ALKANOL OR ALKYNNOL OR ALKENOL OR UREA OR GUANIDINE OR (ALKNAOIC

ACID) OR GLYCOL OR POLYALCOHOL

> s 15 (p) 16  
8           0 L5 (P) L6  
  
> s 15 (p) 17  
9           527 L5 (P) L7  
  
> s 19 (p) impurit?  
10           0 L9 (P) IMPURIT?  
  
> s 19 (p) elut?  
11           103 L9 (P) ELUT?  
  
> duplicate remove l11  
DUPLICATE PREFERENCE IS 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA'  
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n  
PROCESSING COMPLETED FOR L11  
12           37 DUPLICATE REMOVE L11 (66 DUPLICATES REMOVED)  
  
> s 19 (p) (negative charge)  
13           0 L9 (P) (NEGATIVE CHARGE)  
  
> d 112 1-37 ibib abs  
  
12 ANSWER 1 OF 37 CAPLUS COPYRIGHT 2003 ACS  
CESSION NUMBER: 2002:637703 CAPLUS  
DOCUMENT NUMBER: 137:181920  
TITLE: Method for purifying the Helicobacter adhesin-like protein A (AlpA)  
INVENTOR(S): Fourrichon, Laurence; Lissolo, Ling; Pitiot, Olivier  
PATENT ASSIGNEE(S): Merieux Oravax, Fr.  
SOURCE: PCT Int. Appl., 22 pp.  
DOCUMENT TYPE: Patent  
LANGUAGE: French  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:  
  

| PATENT NO.  | KIND   | DATE   | APPLICATION NO. | DATE       |
|---|--|--|-----------------|------------|
| WO 2002064622   | A1   | 20020822   | WO 2002-FR355   | 20020130   |
| W:  | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |  |                 |            |
| RW:   | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG   |  |                 |            |
| FR 2820424  | A1   | 20020809   | FR 2001-1499    | 20010205   |
| PRIORITY APPLN. INFO.:  |  |  | FR 2001-1499    | A 20010205 |
| 3 The invention concerns a method for ***purifying*** the Helicobacter adhesin-like ***protein*** A (AlpA) which consists in: (i) contacting an AlpA prepn. and 2.5 to 3.5 M of ***guanidine*** with a hydrophobic interaction chromatog. material, so that the AlpA is adsorbed on the material; and (ii) ***eluting*** the AlpA with a soln. contg. 3.5 to 4.5 M of ***guanidine***. The AlpA prepn. to be ***purified*** can be in particular derived from an E. coli culture capable of expressing AlpA in a high-level recombinant form, rAlpA being in the form of inclusion bodies, the latter being recovered and solubilized in the presence of ***guanidine***, and optionally ammonium sulfate-ptd. for the purpose of preliminary ***purifn***. The hydrophobic interaction chromatog. can be followed up by an ***anion*** ***exchange*** ***chromatog***. in the presence of 8 M of ***urea***. |  |  |                 |            |
| REFERENCE COUNT:  | 7  | THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT |                 |            |

CUMENT NUMBER: 20290831 PubMed ID: 10828960  
TITLE: Monoclonal antibody light chain with prothrombin activity.  
UTHOR: Thiagarajan P; Dannenbring R; Matsuura K; Tramontano A;  
RPORATE SOURCE: Gololobov G; Paul S  
Departments of Internal Medicine and Pathology and  
Laboratory Medicine, Center for Chemical Immunology,  
University of Texas-Houston Medical School, 77030, USA..  
Perumal.Thiagarajan@uth.tmc.edu

NTRACT NUMBER: AI 31268 (NIAID)

HL 44126 (NHLBI)

HL 65096 (NHLBI)

JRCE: BIOCHEMISTRY, (2000 May 30) 39 (21) 6459-65.  
Journal code: 0370623. ISSN: 0006-2960.

B. COUNTRY: United States

CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

NGUAGE: English

LE SEGMENT: Priority Journals

TRY MONTH: 200007

TRY DATE: Entered STN: 20000720

Last Updated on STN: 20000720

Entered Medline: 20000711

Prothrombin is the precursor of thrombin, a central enzyme in coagulation. Autoantibodies to prothrombin are associated with thromboembolism, but the mechanisms by which the antibodies modulate the coagulation processes are not understood. We screened a panel of 34 monoclonal antibody light chains

\*\*\*isolated\*\*\* from patients with multiple myeloma for prothrombinase activity by an electrophoresis method. Two light chains with the activity were identified, and one of the light chains was characterized further. The prothrombinase activity \*\*\*eluted\*\*\* from a gel-filtration column run in denaturing solvent (6 M \*\*\*guanidine\*\*\* hydrochloride) at the characteristic positions of the light chain dimer and monomer. A constant level of catalytic activity was observed across the width of the light chain monomer peak, assessed as the cleavage of IEGR-methylcoumarinamide, a \*\*\*peptide\*\*\* substrate corresponding to residues 268-271 of prothrombin. Hydrolysis of this \*\*\*peptide\*\*\* by the light chain was saturable and consistent with Michaelis-Menten-Henri kinetics ( $K(m)$  103 microM;  $k(cat)$  of  $2.62 \times 10(-)(2)/min$ ). Four cleavage sites in prothrombin were identified by N-terminal sequencing of the fragments:

Arg(155)-Ser(156), Arg(271)-Thr(272), Arg(284)-Thr(285), and Arg(393)-Ser(394). The light chain did not cleave radiolabeled albumin, thyroglobulin, and annexin V under conditions that readily permitted detectable prothrombin cleavage. Two prothrombin fragments ( $M(r)$  55 000 and 38 000), were \*\*\*isolated\*\*\* by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\*

\*\*\*chromatography\*\*\* and were observed to cleave a thrombin substrate, tosyl-GPR-nitroanilide. Conversion of fibrinogen to fibrin was accelerated by the prothrombin fragments generated by the light chain. These finding suggest a novel mechanism whereby antibodies can induce a procoagulant state, i.e., prothrombin activation via cleavage of the molecule.

2 ANSWER 3 OF 37 MEDLINE

DUPPLICATE 2

CESSION NUMBER: 2002257174 MEDLINE  
CUMENT NUMBER: 21991123 PubMed ID: 11996098  
TITLE: Proteoglycans of human umbilical cord arteries.  
UTHOR: Gogiel T; Jaworski S  
RPORATE SOURCE: Department of Biochemistry, Medical Academy of Bialystok,  
Poland.. tgogiel@amb.ac.bialystok.pl  
ACTA BIOCHIMICA POLONICA, (2000) 47 (4) 1081-91.  
Journal code: 14520300R. ISSN: 0001-527X.

B. COUNTRY: Poland  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 200206  
TRY DATE: Entered STN: 20020509  
Last Updated on STN: 20020625  
Entered Medline: 20020624

3 Proteoglycans (PGs) were dissociatively extracted from human umbilical cord arteries (UCAs) with 4 M \*\*\*guanidine\*\*\* hydrochloride containing Triton X-100 and protease inhibitors, \*\*\*purified\*\*\* by Q-Sepharose \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* - \*\*\*chromatography\*\*\* and lyophilized. They were analysed by gel filtration, SDS/PAGE and agarose gel

electrophoresis before and after treatment with chondroitinase ABC. It was found that the PG preparation was especially enriched in chondroitin/dermatan sulphate PGs. The predominant PG fraction included small PGs that emerged from Sepharose CL-2B with Kav = 0.74. Their molecular mass, estimated by SDS/PAGE, was 160-200 kDa and 90-150 kDa, i.e. it was typical for biglycan and decorin, respectively. Treatment with chondroitinase ABC yielded the core \*\*\*proteins\*\*\* of 45 and 47 kDa, characteristic for both small PGs. Remarkable amounts of the 45 kDa

\*\*\*protein\*\*\* were detected in non-treated PG samples, suggesting the presence of free core \*\*\*proteins\*\*\* of biglycan and decorin. Large PGs were present in lower amounts. In intact form they were \*\*\*eluted\*\*\* from Sepharose CL-2B with Kav = 0.17 and 0.43. Digestion with chondroitinase ABC yielded the core \*\*\*proteins\*\*\* with a molecular mass within the range of 180-360 kDa but predominant were the bands of 200, 250 and 360 kDa. The large PGs probably represent various forms of versican or perlecan bearing chondroitin sulphate chains.

2 ANSWER 4 OF 37 MEDLINE  
CESSION NUMBER: 2001434257 MEDLINE  
DOCUMENT NUMBER: 21094863 PubMed ID: 11162736  
TITLE: A new purification method for overproduced proteins sensitive to endogenous proteases.  
THOR: Saijo-Hamano Y; Namba K; Oosawa K  
CORPORATE SOURCE: Protonic NanoMachine Project, ERATO, JST, 1-7 Hikaridai, Seika 619-0237, Japan.  
URCE: JOURNAL OF STRUCTURAL BIOLOGY, (2000 Nov) 132 (2) 142-6.  
Journal code: 9011206. ISSN: 1047-8477.

B. COUNTRY: United States  
CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 200108  
TRY DATE: Entered STN: 20010806  
Last Updated on STN: 20010806  
Entered Medline: 20010802

Proteolysis is a major problem in \*\*\*purification\*\*\* of overproduced \*\*\*proteins\*\*\* for structural studies. We developed a new method to avoid proteolysis of the products even in cases where popular protease inhibitors do not work effectively. When we cloned FlgF, a flagellar rod \*\*\*protein\*\*\*, from *Salmonella typhimurium* and overproduced it in *Escherichia coli*, FlgF was highly susceptible to cleavage by endogenous proteases after cell disruption even in the presence of various protease inhibitors. However, FlgF was not digested when the cells were disrupted in the presence of \*\*\*urea\*\*\*, which allowed us to develop the following new \*\*\*purification\*\*\* procedure. After cell disruption in the presence of \*\*\*urea\*\*\* and removal of the cell debris, the supernatant was passed through tandem-connected cation- and \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*columns\*\*\*. Proteases were trapped in the cation-exchange column, and protease-free FlgF was \*\*\*eluted\*\*\* from the disconnected \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*column\*\*\*. This gave a stable full-length product suitable for crystallization trials. The key procedures are cell disruption in the presence of \*\*\*urea\*\*\* and linked ion-exchange chromatography to quickly remove proteases as well as \*\*\*urea\*\*\*. This fast and simple method can be applied to \*\*\*purification\*\*\* of other overproduced \*\*\*proteins\*\*\* that are very sensitive to proteolysis.

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2 ANSWER 5 OF 37 CAPLUS COPYRIGHT 2003 ACS  
CESSION NUMBER: 1999:235191 CAPLUS  
DOCUMENT NUMBER: 131:41348  
TITLE: Purification and properties of a cholesterol ester hydrolase from rat liver microsomes  
UTHOR(S): Cristobal, Susana; Ochoa, Begona; Fresnedo, Olaz  
CORPORATE SOURCE: Department of Physiology, University of the Basque Country Medical School, Bilbao, 48080, Spain  
OURCE: Journal of Lipid Research (1999), 40(4), 715-725  
UBLISHER: Lipid Research, Inc.  
DOCUMENT TYPE: Journal  
NGUAGE: English

female rat liver microsomes, and some structural, immunol., kinetic, and regulatory properties of the enzyme that distinguish microsomal CEH from other hepatic cholesteryl ester-splitting enzymes are reported. CEH was \*\*\*purified\*\*\* 12.4-fold from re- \*\*\*isolated\*\*\* microsomes using sequential solubilization by sonication, polyethylene \*\*\*glycol\*\*\* pptn., fractionation with hydroxylapatite, \*\*\*anion\*\*\* \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* ., and chromatog. on hydroxylapatite, with an overall yield of 3.2%. CEH activity was \*\*\*purified\*\*\* 141-fold over nonspecific esterase activity and 56-fold over triacylglycerol lipase activity. In sharp contrast to most esterases and lipases, CEH did not bind to Con A-Sepharose and heparin-Sepharose. After PAGE, the \*\*\*purified\*\*\* enzyme exhibited 2 Ag-stained bands, but only the \*\*\*protein\*\*\* electro- \*\*\*eluted\*\*\* from the low-mobility band had CEH activity. Affinity- \*\*\*purified\*\*\* polyclonal antibodies raised to electro- \*\*\*eluted\*\*\* CEH inhibited 90% of the activity of liver microsomal CEH and reacted with a 106-kDa \*\*\*protein\*\*\* band on Western blot anal. This 106-kDa CEH contained a unique N-terminal amino acid sequence. The \*\*\*purified\*\*\* enzyme had optimal activity at pH 6 and no taurocholate requirement, and was inhibited by the serine active site inhibitor, phenylmethylsulfonyl fluoride, and by free SH group-specific reagents. It hydrolyzed cholesteryl oleate much more efficiently than triolein, and its hydrolytic activity with p-nitrophenyl acetate was higher than with p-nitrophenyl butyrate. Thus, the results indicate that rat liver microsomes contain a bile salt-independent catalytic \*\*\*protein\*\*\* that is relatively specific for cholesteryl ester hydrolysis.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

2 ANSWER 6 OF 37 MEDLINE DUPLICATE 4  
SESSION NUMBER: 1999133646 MEDLINE  
CUMENT NUMBER: 99133646 PubMed ID: 9950147  
TITLE: Purification and characterization of a complex from placental syncytiotrophoblast microvillous membranes which inhibits the proliferation of human umbilical vein endothelial cells.  
AUTHOR: Kertesz Z; Hurst G; Ward M; Willis A C; Caro H; Linton E A; Sargent I L; Redman C W  
CORPORATE SOURCE: Nuffield Department of Obstetrics and Gynaecology, University of Oxford, UK.. zkertesz@radius.jr2.ox.ac.uk  
OURCE: PLACENTA, (1999 Jan) 20 (1) 71-9.  
JOURNAL CODE: Journal code: 8006349. ISSN: 0143-4004.  
SUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 199903  
TRY DATE: Entered STN: 19990413  
Last Updated on STN: 20021030  
Entered Medline: 19990329

3 The signs of pre-eclampsia are thought to arise from maternal endothelial dysfunction caused by circulating factors of placental origin. Syncytiotrophoblast microvillous membranes (STBM) cause endothelial disruption and inhibit proliferation in vitro. Significantly increased amounts of STBM can be detected in blood from pre-eclamptic women and could contribute to endothelial dysfunction in vivo. This study

\*\*\*purified\*\*\* a complex from STBM which inhibits the proliferation of cultured human endothelial cells. Integral membrane \*\*\*proteins\*\*\* were solubilized with sucrose monolaurate. \*\*\*Anion\*\*\*

\*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* yielded two peaks of anti-proliferative activity. Only the second peak was specific to STBM and was subjected to further separation by Sephadryl S-200 gel filtration chromatography (GFC). A single peak of specific activity \*\*\*eluted\*\*\* close to the void volume, at a position unaltered by added denaturing agents, guanidium chloride or \*\*\*urea\*\*\*. On Sephadryl S-300 GFC, two peaks were obtained of 410 and 820 kDa, with similar anti-proliferative activity and \*\*\*protein\*\*\* components (by SDS-polyacrylamide gel electrophoresis). The major \*\*\*protein\*\*\* bands were as integrins alpha5 and alpha v, dipeptidyl peptidase IV, alpha-actinin, transferrin, transferrin \*\*\*receptor\*\*\*, placental alkaline phosphatase and monoamine oxidase A.

ANSWER 7 OF 37

MEDLINE

DUPLICATE 5

SSION NUMBER: 1998334669 MEDLINE  
 MENT NUMBER: 98334669 PubMed ID: 9668118  
 JE: Identification and characterization of a bovine neurite growth inhibitor (bNI-220).  
 IOR: Spillmann A A; Bandtlow C E; Lottspeich F; Keller F; Schwab M E  
 PORATE SOURCE: Brain Research Institute, University of Zurich and Swiss Federal Institute of Technology Zurich, August Forelstrasse 1, 8029 Zurich, Switzerland.  
 RCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jul 24) 273 (30) 19283-93.  
 Journal code: 2985121R. ISSN: 0021-9258.  
 COUNTRY: United States  
 UMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 GUAGE: English  
 E SEGMENT: Priority Journals  
 RY MONTH: 199808  
 RY DATE: Entered STN: 19980828  
 Last Updated on STN: 19980828  
 Entered Medline: 19980820

The poor axonal regeneration that follows lesions of the central nervous system (CNS) is crucially influenced by the local CNS tissue environment through which neurites have to grow. In addition to an inhibitory role of the glial scar, inhibitory substrate effects of CNS myelin and oligodendrocytes have been demonstrated. Several \*\*\*proteins\*\*\* including NI-35/250, myelin-associated glycoprotein, tenascin-R, and NG-2 have been described to have neurite outgrowth inhibitory or repulsive properties in vitro. Antibodies raised against NI-35/250 (monoclonal antibody IN-1) were shown to partially neutralize the growth inhibitory effect of CNS myelin and oligodendrocytes, and to result in long distance fiber regeneration in the lesioned adult mammalian CNS in vivo. We report here the \*\*\*purification\*\*\* of a myelin \*\*\*protein\*\*\* to apparent homogeneity from bovine spinal cord which exerts a potent neurite outgrowth inhibitory effect on PC12 cells and chick dorsal root ganglion cells, induces collapse of growth cones of chick dorsal root ganglion cells, and also inhibits the spreading of 3T3 fibroblasts. These activities could be neutralized by the monoclonal antibody IN-1. The \*\*\*purification\*\*\* procedure includes detergent solubilization, \*\*\*anion\*\*\* \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\*, gel filtration, and \*\*\*elution\*\*\* from high resolution SDS-polyacrylamide gel electrophoresis. The active \*\*\*protein\*\*\* has a molecular mass of 220 kDa and an isoelectric point between 5.9 and 6.2. Its inhibitory activity is sensitive to protease treatment and resists harsh treatments like 9 M \*\*\*urea\*\*\* or short heating. Glycosylation is, if present at all, not detectable. Microsequencing resulted in six \*\*\*peptides\*\*\* and strongly suggests that this \*\*\*proteins\*\*\* is novel.

2 ANSWER 8 OF 37 CAPLUS COPYRIGHT 2003 ACS

CESSION NUMBER: 1997:416857 CAPLUS  
 DOCUMENT NUMBER: 127:39795  
 TLE: Anion exchange process for the purification of Factor VIII  
 JVVENTOR(S): Bhattacharya, Prabir; Motokubota, Toshiharu; Fedalizo, Norman M.  
 ENT ASSIGNEE(S): Alpha Therapeutic Corporation, USA  
 URCE: PCT Int. Appl., 29 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 ANGUAGE: English  
 MILY ACC. NUM. COUNT: 1  
 ENT INFORMATION:

| PATENT NO.  | KIND | DATE     | APPLICATION NO. | DATE     |
|---|------|----------|-----------------|----------|
| WO 9717370  | A1   | 19970515 | WO 1996-US17806 | 19961107 |
| W: CA, JP   |      |          |                 |          |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE                    |      |          |                 |          |
| US 5659017  | A    | 19970819 | US 1995-554724  | 19951107 |
| RIORITY APPLN. INFO.: A new method for ***purifying*** Factor VIII complex from an impure |      |          | US 1995-554724  | 19951107 |
| The invention is  |      |          |                 |          |

dissolved in a heparin soln. Factor VIII complex is initially \*\*\*purified\*\*\* by polyethylene \*\*\*glycol\*\*\* pptn. The Factor VIII contg. supernatant collected after the pptn. is loaded into an \*\*\*anion\*\*\* \*\*\*exchange\*\*\* \*\*\*column\*\*\* that has a quaternary amino Et group. The Factor VIII complex is then \*\*\*eluted\*\*\* from the column with a buffer comprising from about 0.14M to about 0.20M CaCl<sub>2</sub>. The final step in the \*\*\*purifn\*\*\* is to ppt. the Factor VIII complex in the presence of glycine and sodium chloride. The pptd. Factor VIII complex is then reconstituted and stabilized. The reconstituted Factor VIII complex can then be lyophilized and dry heated to obtain a final Factor VIII product.

2 ANSWER 9 OF 37 CAPLUS COPYRIGHT 2003 ACS

CESSION NUMBER: 1997:695207 CAPLUS  
CUMENT NUMBER: 127:344221  
TITLE: Characteristics of chondroitin sulfated proteoglycans in the matrix phase of human alveolar bone  
THOR(S): Zhao, Hu; Suzuki, Naoto; Maeno, Masao; Katayama, Ichiro; Arai, Toshiyuki; Otsuka, Kichibee  
RPORATE SOURCE: Department of Biochemistry, Nihon University School of Dentistry, Kanda-surugadai, 101, Japan  
URCE: Journal of Hard Tissue Biology (1997), 6(1), 1-9  
CODEN: JHTBFF; ISSN: 1341-7649  
BLISHER: Japanese Society of Hard Tissue Research & Technology  
CUMENT TYPE: Journal  
NGUAGE: English

This study demonstrates the types and characteristics of chondroitin sulfated proteoglycans (CSPGs) in the matrix phase of human alveolar bone (HAB) extd. with \*\*\*guanidine\*\*\* -HCl from EDTA-demineralized HAB fragments. Different CSPGs including dermatan sulfated PG were analyzed and identified using three monoclonal antibodies in combination with different chondroitinase digestions. The PGs were partially \*\*\*purified\*\*\* by gel filtration and then \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*column\*\*\* chromatog. under denaturing conditions. The CSPGs in each \*\*\*eluted\*\*\* fraction from the columns were detected by ELISA using a monoclonal antibody (MAb) 2B6 in combination with chondroitinase ABC digestion. 2B6-Reactive fractions of the \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*column\*\*\* were concd. in three fractions. The types of glycosaminoglycan (GAG) chains of CSPGs were detd. by Western blotting using antibodies against GAG stubs on a core \*\*\*protein\*\*\* after enzyme digestion. The main CSPGs in the matrix phase of HAB were comprised of dermatan sulfated PG contg. a 45 kDa core \*\*\*protein\*\*\*. There were also small amts. of chondroitin non-sulfated PGs which consisted of 45 and 110 kDa core \*\*\*proteins\*\*\*, but no chondroitin 6-sulfated PG was detected.

2 ANSWER 10 OF 37 MEDLINE

DUPPLICATE 6

CESSION NUMBER: 95357413 MEDLINE  
CUMENT NUMBER: 95357413 PubMed ID: 7630943  
TITLE: Purification of NAD-dependent mannitol dehydrogenase from celery suspension cultures.  
JTHOR: Stoop J M; Williamson J D; Conkling M A; Pharr D M  
RPORATE SOURCE: Department of Horticultural Science, North Carolina State University, Raleigh 27695-7609, USA.  
URCE: PLANT PHYSIOLOGY, (1995 Jul) 108 (3) 1219-25.  
Journal code: 0401224. ISSN: 0032-0889.

JB. COUNTRY: United States  
CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
ILE SEGMENT: Priority Journals  
TRY MONTH: 199509  
TRY DATE: Entered STN: 19950921  
Last Updated on STN: 20020420  
Entered Medline: 19950905

3 Mannitol dehydrogenase, a mannitol:mannose 1-oxidoreductase, constitutes the first enzymatic step in the catabolism of mannitol in nonphotosynthetic tissues of celery (*Apium graveolens L.*). Endogenous regulation on the enzyme activity in response to environmental cues is critical in modulating tissue concentration of mannitol, which, importantly, contribute to stress tolerance of celery. The enzyme was \*\*\*purified\*\*\* to homogeneity from celery suspension cultures grown on

\*\*\*purified\*\*\* 589-fold to a specific activity of 365  $\mu$ mol $^{-1}$  mg $^{-1}$   
\*\*\*protein\*\*\* with a 37% yield of enzyme activity present in the crude extract. A highly efficient and simple \*\*\*purification\*\*\* protocol was developed involving polyethylene \*\*\*glycol\*\*\* fractionation, diethylaminoethyl- \*\*\*anion\*\*\* - \*\*\*exchange\*\*\*  
\*\*\*chromatography\*\*\*, and NAD-agarose affinity chromatography using NAD gradient \*\*\*elution\*\*\*. Sodium dodecylsulfate gel electrophoresis of the final preparation revealed a single 40-kD \*\*\*protein\*\*\*. The molecular mass of the native \*\*\*protein\*\*\* was determined to be approximately 43 kD, indicating that the enzyme is a monomer. Polyclonal antibodies raised against the enzyme inhibited enzymatic activity of \*\*\*purified\*\*\* mannitol dehydrogenase. Immunoblots of crude \*\*\*protein\*\*\* extracts from mannitol-grown celery cells and sink tissues of celery, celeriac, and parsley subjected to sodium dodecyl sulfate gel electrophoresis showed a single major immuno-reactive 40-kD \*\*\*protein\*\*\*.

ANSWER 11 OF 37 CAPLUS COPYRIGHT 2003 ACS

SESSION NUMBER: 1996:111382 CAPLUS  
DOCUMENT NUMBER: 124:141355  
TITLE: In vitro biosynthesis of caffeine; the stability of N-methyltransferase activity in cell-free preparations from liquid endosperm of Coffea arabica  
AUTHOR(S): Gillies, F. M.; Jenkins, G. I.; Ashihara, H.; Crozier, A.  
REPORT SOURCE: Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK  
JOURNAL: Colloque Scientifique International sur le Cafe, [Comptes Rendus] (1995), 16th(Vol. 2, Seizieme Colloque Scientifique International sur le Cafe, 1995, Vol. 2), 599-605  
CODEN: CICRD8  
PUBLISHER: Association Scientifique Internationale du Cafe  
DOCUMENT TYPE: Journal  
LANGUAGE: English

A cell-free system has been developed from C. arabica that is a rich source of the N-methyltransferase activity which catalyzes the transfer of the Me group from S-adenosyl-L-methionine to methylxanthines producing caffeine. \*\*\*Purifn\*\*\* . of the enzyme by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . results in low activity yields. Part of the reason was found to be inhibition of the N-methyltransferase activity by KCl and NaCl which are used to \*\*\*elute\*\*\* the \*\*\*protein\*\*\* during \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . The enzyme was found to have a half life at 4 degree C of approx. 90 min. An extensive study of a wide range of protease inhibitors failed to show any effective stabilization of activity suggesting that the losses are not due to the action of endogenous proteases in the ext. The stability of the enzyme has been shown to be improved substantially with the incorporation of 20% (vol./vol.) glycerol or 20% (vol./vol.) ethylene \*\*\*glycol\*\*\* in the buffers used. Incorporation of 20% (vol./vol.) glycerol in buffers during \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . resulted in 54 - 78% yield of N-methyltransferase activity and a ca. 10-20 fold \*\*\*purifn\*\*\* .

2 ANSWER 12 OF 37 MEDLINE DUPLICATE 7  
SESSION NUMBER: 95102513 MEDLINE  
DOCUMENT NUMBER: 95102513 PubMed ID: 7804133  
TITLE: Rapid purification of tRNA(Lys) from rat liver.  
AUTHOR: Kumar A M; Vulimiri S V; Nayak R  
REPORT SOURCE: Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore.  
JOURNAL: BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, (1994 Aug) 33 (6) 1081-9.  
Journal code: 9306673. ISSN: 1039-9712.  
COUNTRY: Australia  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199501  
ENTRY DATE: Entered STN: 19950215  
Last Updated on STN: 19980206

Fast \*\*\*protein\*\*\* liquid chromatography (FPLC) system using Mono Q (HR 5/5) \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*column\*\*\* chromatography followed by highly cross-linked \*\*\*urea\*\*\* -polyacrylamide gel electrophoresis ( \*\*\*urea\*\*\* -PAGE) was used for the \*\*\*purification\*\*\* of lysine-specific tRNA (tRNA(Lys)) from rat liver. Crude tRNA from rat liver was fractionated with a linear gradient of NaCl (0.3-0.8 M) in triethanolamine-HCl buffer, pH 4.5, and the activity of tRNA(Lys) was found to \*\*\*elute\*\*\* between 0.51 and 0.57 M NaCl. Using this concentration range of NaCl, tRNA(Lys) was refractionated on the same column with a shallow gradient, where a single peak of tRNA(Lys) activity was obtained. tRNA(Lys)-rich fractions recovered from the second run were electrophoretically separated on 16% polyacrylamide-7 M \*\*\*urea\*\*\* gel into one major band and three minor bands. The major band showed a specific activity of 997 pmols/A260 U for tRNALys with a 43-fold \*\*\*purification\*\*\* and approximately 17% recovery. The minor bands displayed negligible or no activity for lysine. tRNA(Lys) obtained by this method was found to be homogeneous by competitive aminoacylation. The advantages of FPLC followed by \*\*\*urea\*\*\* -PAGE in the \*\*\*purification\*\*\* of an amino acid-specific tRNA over conventional column chromatography are discussed.

2 ANSWER 13 OF 37 MEDLINE DUPLICATE 8  
SESSION NUMBER: 95071402 MEDLINE  
CUMENT NUMBER: 95071402 PubMed ID: 7980550  
TITLE: Rapid isolation of G alpha 13 from bovine brain membranes: supportive effect of ethylene glycol.  
THOR: Harhammer R; Nurnberg B; Spicher K; Schultz G  
RPORTATE SOURCE: Institut fur Pharmakologie, Universitatsklinikum Rudolf Virchow, Freie Universitat Berlin, Germany.  
URCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1994 Oct 28) 204 (2) 835-40.  
Journal code: 0372516. ISSN: 0006-291X.  
B. COUNTRY: United States  
CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 199411  
TRY DATE: Entered STN: 19950110  
Last Updated on STN: 20000303  
Entered Medline: 19941130

G13 belongs to the G12-subfamily of heterotrimeric regulatory G- \*\*\*proteins\*\*\*. Employing specific antibodies, we \*\*\*isolated\*\*\* G alpha 13 from bovine brain by a four-step \*\*\*purification\*\*\* protocol combining conventional and affinity chromatography. The use of ethylene \*\*\*glycol\*\*\* as a protective agent influenced the \*\*\*elution\*\*\* properties of G alpha 13 markedly. Only in the presence of ethylene \*\*\*glycol\*\*\* (30% v/v) a clear separation of G alpha 13 from other G- \*\*\*proteins\*\*\* was achieved during the initial \*\*\*anion\*\*\* \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\*. This allowed \*\*\*isolation\*\*\* of G alpha 13 by subunit exchange chromatography on beta gamma-agarose. G alpha 13 was only released from immobilized beta gamma-dimers via activation by AMF but not by GTP gamma S, pointing to a poor basal nucleotide exchange of this \*\*\*protein\*\*\*. In contrast, N-terminally truncated G alpha 13 did not bind to immobilized beta gamma-dimers.

12 ANSWER 14 OF 37 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 9  
SESSION NUMBER: 1994:187267 CAPLUS  
DOCUMENT NUMBER: 120:187267  
TITLE: Sucrose-phosphate synthase is regulated via metabolites and protein phosphorylation in potato tubers, in a manner analogous to the enzyme in leaves  
UTHOR(S): Reimholz, Ralph; Geigenberger, Peter; Stitt, Mark  
RPORTATE SOURCE: Univ. Bayreuth, Bayreuth, D-95447, Germany  
URCE: Planta (1994), 192(4), 480-8  
CODEN: PLANAB; ISSN: 0032-0935  
DOCUMENT TYPE: Journal  
NGUAGE: English

3 Sucrose-phosphate synthase (SPS) was \*\*\*purified\*\*\* 40-fold from stored potato (*Solanum tuberosum L.*) tubers to a final specific activity of 33-70 nkat (mg \*\*\*protein\*\*\*)-1 via batch \*\*\*elution\*\*\* from diethylaminoethyl (DEAE)-sephadex, polyethylene \*\*\*glycol\*\*\* (PEG)

Immunoblotting revealed a major and a minor band with mol. wts. of 124.8 kDa and 133.5 kDa, resp. Both bands were also present in exts. prep'd. in boiling SDS to exclude proteolysis. No smaller \*\*\*polypeptides\*\*\* were seen, except when the preps. were incubated before application on a polyacrylamide gel. The enzyme prepn. was activated by glucose-6-phosphate and inhibited by inorg. phosphate. Both effectors had a large effect on the Km (fructose-6-phosphate) and the Km (uridine-5-diphosphoglucose) with phosphate acting antagonistically to glucose-6-phosphate. Preincubation of potato slices with low concns. of okadaic acid or microcystin resulted in a 3-4-fold decrease in the activity of SPS when the tissue was subsequently extd. and assayed. The decrease was esp. marked when the assay contained low concns. of substrates and glucose-6-phosphate, and inorg. phosphate was included. Preincubation with mannose or in high osmoticum resulted in an increase of SPS activity. Analogous changes were obsd. in germinating Ricinus communis L. seedlings. After preincubation of the cotyledons in glucose, high SPS activity could be measured, whereas okadaic acid, omission of glucose, or addn. of phosphate or sucrose led to a large decrease of SPS activity in the selective assay. It is argued that SPS from non-photosynthetic tissues is regulated by metabolites and by \*\*\*protein\*\*\* phosphorylation in an analogous manner to the leaf enzyme.

? ANSWER 15 OF 37 MEDLINE DUPLICATE 10  
SESSION NUMBER: 94163329 MEDLINE  
DOCUMENT NUMBER: 94163329 PubMed ID: 8118550  
TITLE: Use of polyethylene glycol and high-performance liquid chromatography for preparative separation of Aspergillus ficuum acid phosphatases.  
AUTHOR: Hamada J S  
CORPORATE SOURCE: US Department of Agriculture, Southern Regional Research Center, New Orleans, LA 70179.  
JOURNAL: JOURNAL OF CHROMATOGRAPHY A, (1994 Jan 14) 658 (2) 371-80.  
Journal code: 9318488.  
B. COUNTRY: Netherlands  
CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 199404  
TRY DATE: Entered STN: 19940412  
Last Updated on STN: 19940412  
Entered Medline: 19940407

\*\*\*Proteins\*\*\* of Aspergillus ficuum culture filtrate were sequentially fractionated with 4, 9, 15, 19, 24, 30 and 36% polyethylene glycol\*\*\* (PEG) into seven acid phosphatases (APases) with 93% and 52% overall recoveries of activity and \*\*\*protein\*\*\*, respectively. Crude extract was also separated into seven APase peaks on a 30 cm x 2.5 cm I.D. \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*column\*\*\* using 0.1 M Tris-HCl (pH 8.0) and a 0-0.4 M KCl gradient as the eluent, but their resolution was incomplete. However, when individual PEG precipitates were injected on to the column, each APase was \*\*\*eluted\*\*\* in a single, large peak resulting in 85% recovery and fifteen-fold \*\*\*purification\*\*\* of APase activity over the PEG precipitates. Use of PEG prior to HPLC separations also reduced the separation time to half and allowed a tenfold increase in sample load with complete resolution. The APases in PEG fractions and their corresponding HPLC peaks varied significantly in their kinetic parameters, including substrate specificity and pH optimum. The method developed is most beneficial for the \*\*\*isolation\*\*\* of these closely related APases from microbial or other sources for further molecular biology studies.

.2 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 11  
SESSION NUMBER: 1994:161885 CAPLUS  
DOCUMENT NUMBER: 120:161885  
TITLE: Semipreparative isolation of bovine casein components by high-performance liquid chromatography  
AUTHOR(S): Ng-Kwai-Hang, K. F.; Chin, Dong  
CORPORATE SOURCE: Dep. Anim. Sci., McGill Univ., Sté Anne de Bellevue, QC, H9X 3V9, Can.  
JOURNAL: International Dairy Journal (1994), Volume Date 1993, 4(2), 99-110  
CODEN: IDAJE6; ISSN: 0958-6946

NGUAGE: English  
An HPLC technique using a \*\*\*protein\*\*\* PAK DEAE-15 HR \*\*\*anion\*\*\* \*\*\*exchange\*\*\* \*\*\*column\*\*\* was developed for the \*\*\*isolation\*\*\* of electrophoretically pure .kappa.-casein, .beta.-casein, .alpha.s2-casein, and .alpha.s1-casein from 250 mg of whole casein. A 20 mM tris buffer in 4.5 M \*\*\*urea\*\*\* at pH 7.0 and a convex NaCl gradient from 0 to 0.15 M, followed by a concave NaCl gradient from 0.15 to 0.4 M provided the optimum conditions for sepn. of the casein components. The actual sepn. time of the 4 major caseins was less than 1 h. After allowing time for column regeneration and equilibration, whole casein samples for sepn. could be loaded into the system every 2 h. Quant. measurements of the \*\*\*eluted\*\*\* fractions correlated well with known literature values and all the casein components were accounted for.

2 ANSWER 17 OF 37 MEDLINE DUPLICATE 12  
SESSION NUMBER: 94032311 MEDLINE  
CUMENT NUMBER: 94032311 PubMed ID: 8218256  
TITLE: Secondary structure analysis of purified functional CHIP28 water channels by CD and FTIR spectroscopy.  
THOR: Van Hoek A N; Wiener M; Bicknese S; Miercke L; Biwersi J; Verkman A S  
RPORTER SOURCE: Department of Medicine, University of California, San Francisco 94143.  
NTRACT NUMBER: DK35124 (NIDDK)  
DK43840 (NIDDK)  
HL42368 (NHLBI)

+  
URCE: BIOCHEMISTRY, (1993 Nov 9) 32 (44) 11847-56.  
Journal code: 0370623. ISSN: 0006-2960.  
B. COUNTRY: United States  
CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 199312  
TRY DATE: Entered STN: 19940117  
Last Updated on STN: 19970203  
Entered Medline: 19931216

The integral membrane \*\*\*protein\*\*\* CHIP28 is an important water channel in erythrocytes and kidney tubule epithelia and is a member of a family of channel/pore \*\*\*proteins\*\*\* including the lens \*\*\*protein\*\*\* MIP26. The purposes of this study were to \*\*\*purify\*\*\* functional, delipidated CHIP28 to homogeneity and to determine secondary structure by circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR). CHIP28 was initially \*\*\*purified\*\*\* and delipidated by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* following solubilization of N-lauroylsarcosine-stripped erythrocyte membranes with beta-octylglucoside (OG); MIP26 was initially \*\*\*purified\*\*\* and delipidated by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* following solubilization of \*\*\*urea\*\*\* -stripped bovine lens membranes by monomyristoylphosphatidylcholine. CHIP28 (glycosylated and nonglycosylated) and MIP26 were \*\*\*purified\*\*\* further by high-performance size-exclusion chromatography, \*\*\*eluting\*\*\* in OG as apparent dimers and tetramers, respectively. Proteoliposomes reconstituted with \*\*\*purified\*\*\* CHIP28 were highly water-permeable, with an osmotic water permeability Pf of 0.04 cm/s at 10 degrees C that was inhibited by 0.1 mM HgCl<sub>2</sub>. Proteoliposomes reconstituted with MIP26 had a low Pf of 0.005 cm/s. CD spectra of CHIP28 in OG or in reconstituted proteoliposomes gave a maximum at 193 nm and minima at 208 and 222 nm. Spectral decomposition using \*\*\*protein\*\*\* basis spectra gave 40 +/- 5% alpha-helix and 43 +/- 3% beta-sheet and -turn. HgCl<sub>2</sub> did not affect the CD spectrum of CHIP28. Attenuated total reflectance FTIR of air-dried, membrane-associated CHIP28 gave 38 +/- 5% alpha-helix and 40 +/- 4% beta-sheet and -turn by spectral decomposition of the amide I resonance. For comparison, CD of MIP26 in OG gave 49 +/- 7% alpha-helix and 32 +/- 12% beta-sheet and -turn; FTIR gave 32 +/- 8% alpha-helix and 45 +/- 6% beta-sheet and -turn. Analysis of CHIP28 and MIP26 sequence data by the generalized hydrophobicity method of Jahnig [Jahnig, F. (1990) Trends Biochem. Sci. 15, 93-95] predicted 39-47% alpha-helix and 15-20% beta-structures. These results establish procedures to obtain large quantities of pure CHIP28 and MIP26 in functional forms and provide evidence for multiple membrane-spanning alpha-helices or mixed alpha/beta-domains.

SESSION NUMBER: 1993:54851 CAPLUS

DOCUMENT NUMBER: 118:54851

TITLE: Purification and characterization of  
1-aminocyclopropane-1-carboxylate N-malonyltransferase  
from etiolated mung bean hypocotylsAUTHOR(S): Guo, Lining; Arteca, Richard N.; Phillips, Allen T.;  
Liu, YuCORPORATE SOURCE: Dep. Hortic., Pennsylvania State Univ., University  
Park, PA, 16802, USA

JURCE: Plant Physiology (1992), 100(4), 2041-5

DOCUMENT TYPE: CODEN: PLPHAY; ISSN: 0032-0889

LANGUAGE: English

1-Aminocyclopropane-1-carboxylate (ACC) N-malonyltransferase converts ACC, an immediate precursor of ethylene, to the presumably inactive product malonyl-ACC (MACC). This enzyme plays a role in ethylene prodn. by reducing the level of free ACC in plant tissue. In this study, ACC N-malonyltransferase was \*\*\*purified\*\*\* 3660-fold from etiolated mung bean (*Vigna radiata*) hypocotyls, with a 6% overall recovery. The final specific activity was about 83,000 nmol of MACC formed mg<sup>-1</sup>

\*\*\*protein\*\*\* h<sup>-1</sup>. The five-step \*\*\*purifn\*\*\* protocol consisted of polyethylene \*\*\*glycol\*\*\* fractionation, Cibacron blue 3GA-agarose chromatog. using salt gradient \*\*\*elution\*\*\*, Sephadex G-100 gel filtration, MonoQ \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\*, and Cibacron blue 3GA-agarose chromatog. using malonyl-CoA plus ACC for \*\*\*elution\*\*\*. The mol. mass of the native enzyme detd. by Sephadex G-100 chromatog. was 50 kD. \*\*\*Protein\*\*\* from the final

\*\*\*purifn\*\*\* step showed one major band at 55 kD after sodium dodecyl sulfate polyacrylamide gel electrophoresis, indicating that ACC N-malonyltransferase is a monomer. The mung bean ACC N-malonyltransferase has a pH optimum of 8.0, an apparent Km of 0.5 mM for ACC and 0.2 mM for malonyl-CoA, and an Arrhenius activation energy of 70.29 kJ mol<sup>-1</sup> degree<sup>-1</sup>.

SESSION NUMBER: 92201243 MEDLINE

DOCUMENT NUMBER: 92201243 PubMed ID: 1839382  
TITLE: Identification of the microvillar 110-kDa calmodulin complex (myosin-1) in kidney.AUTHOR: Coluccio L M  
CORPORATE SOURCE: Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

CONTRACT NUMBER: 1R01 GM44211 (NIGMS)

SOURCE: S07 RR05364 (NCRR)  
EUROPEAN JOURNAL OF CELL BIOLOGY, (1991 Dec) 56 (2) 286-94.

Journal code: 7906240. ISSN: 0171-9335.

SUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

COUNTRY MONTH: 199204

COUNTRY DATE: Entered STN: 19920509

Last Updated on STN: 19920509

Entered Medline: 19920430

3 The epithelial layer lining the proximal convoluted tubule of mammalian kidney contains a brush border of numerous microvilli. These microvilli appear in structure to be very similar to the microvilli on epithelial cells of the small intestine. Microvilli found in both the small intestine and the proximal convoluted tubules in kidney have a core bundle of actin filaments bundled by the accessory \*\*\*proteins\*\*\* villin and fimbrin. Along the length of intestinal microvilli, lateral links can be observed to connect the core bundle of actin filaments to the membrane. These cross-bridges are comprised of a 110-kDa calmodulin complex which belongs to a class of single-headed myosin molecules, collectively referred to as myosin-1. We now report that an analogous calmodulin-binding \*\*\*polypeptide\*\*\* of 105 kDa has been identified in rat kidney cortex.

The 105-kDa \*\*\*polypeptide\*\*\* is preferentially found in

\*\*\*purified\*\*\* kidney brush borders, can be extracted with ATP, and co-

\*\*\*elutes\*\*\* with calmodulin on gel filtration and \*\*\*anion\*\*\*

\*\*\*exchange\*\*\* \*\*\*chromatography\*\*\*. Fractions containing the

\*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* exhibit a modest ATPase activity in buffer

containing CaCl<sub>2</sub>. The partially \*\*\*purified\*\*\* 105-kDa \*\*\*polypeptide\*\*\* will bind iodinated calmodulin and will sediment with F-actin in buffer containing ethylene \*\*\*glycol\*\*\* -bis-(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) or Ca<sup>2+</sup>. The addition of ATP partially reverses this association with F-actin. These results indicate that myosin-1, in addition to its presence in intestinal brush borders, is present in the brush border of kidney. We also provide preliminary evidence to indicate that the 105-kDa \*\*\*polypeptide\*\*\* is not restricted to tissues possessing a brush border.

2 ANSWER 20 OF 37 MEDLINE DUPLICATE 15  
ACCESSION NUMBER: 92338871 MEDLINE  
DOCUMENT NUMBER: 92338871 PubMed ID: 1668267  
TITLE: High-yield purification of potato tuber pyrophosphate: fructose-6-phosphate 1-phosphotransferase.  
AUTHOR: Moorhead G B; Plaxton W C  
CORPORATE SOURCE: Department of Biology, Queen's University, Kingston Ontario, Canada.  
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1991 Feb) 2 (1) 29-33.  
SUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199208  
ENTRY DATE: Entered STN: 19920911  
Last Updated on STN: 19920911  
Entered Medline: 19920826

3 The procedure of Yuan et al. (1988, Biochem. Biophys. Res. Commun. 154, 111-117) for the \*\*\*isolation\*\*\* of potato pyrophosphate:fructose-6-phosphate 1-phosphotransferase (PFP) has been modified so that a high yield of homogeneous enzyme could be obtained. Modifications included a lower temperature heat step, a lower percentage initial polyethylene \*\*\*glycol\*\*\* fractionation step (0 to 4%, w/v), stepwise \*\*\*elution\*\*\* following an increase from 30 to 50 mM pyrophosphate during affinity chromatography on Whatman P11 phosphocellulose, \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* using Q-Sepharose "Fast Flow," and gel filtration chromatography with Superose 6 "Prep grade." Our procedure resulted in an overall 42% yield and a final specific activity of 87 mumol fructose 1,6-bisphosphate produced per minute per milligram \*\*\*protein\*\*\*. Rabbit anti-(potato PFP) polyclonal antibodies effectively immunoprecipitated the activity of both the pure enzyme and the enzyme from a crude extract. Western blot analysis demonstrated that the antibodies were monospecific for PFP. A survey of various potato cultivars demonstrated significant differences in PFP activity with respect to fresh weight. This observation should be taken into consideration before any \*\*\*purification\*\*\* of potato PFP is undertaken.

2 ANSWER 21 OF 37 MEDLINE DUPLICATE 16  
ACCESSION NUMBER: 91009234 MEDLINE  
DOCUMENT NUMBER: 91009234 PubMed ID: 2170394  
TITLE: Purification and characterization of glycosyl-phosphatidylinositol-specific phospholipase D.  
AUTHOR: Huang K S; Li S; Fung W J; Hulmes J D; Reik L; Pan Y C; Low M G  
CORPORATE SOURCE: Department of Protein Biochemistry, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.  
CONTRACT NUMBER: GM-35873 (NIGMS)  
GM-40083 (NIGMS)  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 Oct 15) 265 (29) 17738-45.  
Journal code: 2985121R. ISSN: 0021-9258.  
SUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199011  
ENTRY DATE: Entered STN: 19910117  
Last Updated on STN: 19970203  
Entered Medline: 19901121

We have developed a simple immunoaffinity chromatography procedure for the purification of a glycosyl-phosphatidylinositol (GPI)-specific phospholipase D (GPI-PLD) from bovine serum. The enzyme was initially purified by a procedure consisting of 9% polyethylene glycol precipitation, Q Sepharose anion exchange - chromatography, S-300 gel filtration, wheat germ lectin-Sepharose, hydroxylapatite agarose, zinc chelate matrix, Mono Q-high performance liquid chromatography (HPLC), and Superose 12 (gel filtration) HPLC. Using this purified material as immunogen, we generated a panel of monoclonal antibodies. A low affinity antibody was selected for the purification of catalytically active GPI-PLD from bovine serum by immunoaffinity chromatography, followed by wheat germ lectin-Sepharose and Mono Q-fast protein liquid chromatography. The latter method provides a simple purification procedure with an overall yield of 26%. The purified enzyme has an apparent molecular weight of about 100,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and a pI of about 5.6 by isoelectric focusing gel analysis. On Superose 12 HPLC, the material purified by the latter method elutes as a single peak with an apparent molecular weight of 200,000 as determined by protein standards. The enzyme activity is inhibited by [ethylenebis(oxyethylenenitrilo)]tetraacetic acid or 1,10-phenanthroline. Phosphatidic acid is the only 3H-labeled product when [3H]myristate-labeled variant surface glycoprotein is hydrolyzed by the purified enzyme. Amino terminal sequence analysis of the intact 100-kDa protein reveals no strong homology to that of any other known protein. Twelve tryptic peptides derived from the intact protein have been subjected to amino acid sequence analysis. Two of them share sequence homology with each other and with the metal ion binding domains of members of the integrin family. Based upon these criteria, it appears that the purified enzyme is distinct from other phospholipases with specificity for inositol phospholipids.

? ANSWER 22 OF 37 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 17  
SESSION NUMBER: 1991:160378 CAPLUS  
CUMENT NUMBER: 114:160378  
FILE: Two class I aldolases in Klebsormidium flaccidum (Charophyceae): an evolutionary link from chlorophytes to higher plants  
AUTHOR(S): Jacobshagen, Sigrid; Schnarrenberger, Claus  
REPORT SOURCE: Inst. Pflanzenphysiol. Mikrobiol., Freie Univ. Berlin, Berlin, D-1000/33, Germany  
JRC: Journal of Phycology (1990), 26(2), 312-17  
CODEN: JPYLAJ; ISSN: 0022-3646  
CUMENT TYPE: Journal  
LANGUAGE: English

Two fructose-diphosphate aldolase from *K. flaccidum* were purified by affinity elution from phosphocellulose. The 2 enzymes were subsequently sepd. by HPLC on an anion - exchange column (QAE-silica). The aldolase eluting first represented 5% of the total activity; the other aldolase represented the remaining activity. The activity of the enzymes was not reduced by the presence of 1 mM EDTA or increased by 0.1 mM Zn<sup>2+</sup>, establishing their character as class I type (Me<sup>2+</sup> independent) aldolases. The Km values were 1.7 and 34.7 .mu.M for the enzyme eluting first and second, resp., from the QAE-silica column. The subunit mol. masses, as detd. by SDS-PAGE, were 40.5 and 37 kD; the specific activities of the purified enzymes were 7.9 and 24.7 U/mg protein, resp. The 2 aldolases of *K. flaccidum* are homologous to the cytosol and chloroplast specific isoenzymes of higher plants by several criteria and are therefore probably located in the same cellular compartments in *K. flaccidum*. The Km and specific activity for the chloroplast aldolase of *K. flaccidum* are 3 times higher than for the chloroplast aldolase of higher plants, a remarkable difference. Immunotitrn. with specific antisera against the chloroplast aldolase of *Chlamydomonas reinhardtii* and spinach showed that the chloroplast aldolase of *K. flaccidum* was immunochem. intermediate in structure to the resp. aldolases of *C. reinhardtii* and higher plants. *K. flaccidum* is the second species of Charophyceae (besides *Chara foetida*) with 2 class I aldolases as in higher plants whereas 2 species of Chorophyceae have only one class I aldolase and, under some conditions, an addnl. class II (Me<sup>2+</sup> dependent) aldolase. Thus, aldolases may turn out, in addn. to the known enzymes of glycolate to be a novel enzyme

system to evaluate algal evolution along with cytol. features.

ANSWER 23 OF 37 CAPLUS COPYRIGHT 2003 ACS

SESSION NUMBER: 1990:104838 CAPLUS

MENT NUMBER: 112:104838

E: Process for the chromatographic purification of a  
69,000-dalton outer membrane protein of *Bordetella*  
pertussis for vaccines

NTOR(S): Burns, D. L.; Brennan, M. J.; Gould-Kostka, J. L.;  
Manclark, C. R.

INT ASSIGNEE(S): United States Dept. of Health and Human Services, USA

RCE: U. S. Pat. Appl., 14 pp. Avail. NTIS Order No.

PAT-APPL-7-308 864.

CODEN: XAXXAV

JMENT TYPE: Patent

GUAGE: English

ILY ACC. NUM. COUNT: 2

ENT INFORMATION:

| PATENT NO. | KIND | DATE     | APPLICATION NO. | DATE     |
|------------|------|----------|-----------------|----------|
| US 308864  | A0   | 19890715 | US 1989-308864  | 19890210 |
| US 5101014 | A    | 19920331 | US 1989-308864  | 19890210 |

ORITY APPLN. INFO.: US 1989-308864 19890210

The title process involves prep. a \*\*\*protein\*\*\* ext. contg. the  
\*\*\*protein\*\*\*, applying the ext. to an \*\*\*anion\*\*\* - \*\*\*exchange\*\*\*  
\*\*\*column\*\*\* (e.g. DEAE-Sepharose), sepg. the \*\*\*protein\*\*\* from the  
ext. and \*\*\*eluting\*\*\* it with a linear salt gradient, pooling  
fractions contg. the \*\*\*protein\*\*\*, applying the pooled fractions to  
an affinity column contg. a \*\*\*protein\*\*\* -specific binding medium  
(e.g. Affi-Gel Blue), and \*\*\*eluting\*\*\* the \*\*\*purified\*\*\*  
\*\*\*protein\*\*\* (e.g. with .apprx.4 M \*\*\*urea\*\*\*). The  
\*\*\*protein\*\*\* was \*\*\*purified\*\*\* from a \*\*\*protein\*\*\* ext. of B.  
pertussis Bp 353. It bound monoclonal antibody BPE3.

? ANSWER 24 OF 37 MEDLINE

DUPLICATE 18

SESSION NUMBER: 89109147 MEDLINE

CUMENT NUMBER: 89109147 PubMed ID: 2521480

FILE: Analysis of the proteoglycans synthesized by corneal  
explants from embryonic chicken. II. Structural  
characterization of the keratan sulfate and dermatan  
sulfate proteoglycans from corneal stroma.

THOR: Midura R J; Hascall V C

RPORTATE SOURCE: Proteoglycan Chemistry Section, National Institute of  
Dental Research, Bethesda, Maryland 20892.

NTRACT NUMBER: EY05779 (NEI)

URCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jan 25) 264 (3)  
1423-30.

Journal code: 2985121R. ISSN: 0021-9258.

B. COUNTRY: United States

CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

NGUAGE: English

LE SEGMENT: Priority Journals

TRY MONTH: 198903

TRY DATE: Entered STN: 19900308

Last Updated on STN: 19970203

Entered Medline: 19890303

3 Radioisotopically labeled proteoglycans were \*\*\*isolated\*\*\* from a 4 M  
\*\*\*guanidine\*\*\* HCl, 2% Triton X-100 extract of corneal stroma from day  
18 chicken embryos by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\*  
\*\*\*chromatography\*\*\*. Two predominant proteoglycans in the sample were  
separated by octyl-Sepharose chromatography using a gradient  
\*\*\*elution\*\*\* of detergent in 4 M \*\*\*guanidine\*\*\* HCl. One  
proteoglycan had an overall mass of approximately 125 kDa, a single  
dermatan sulfate chain (approximately 85-90% chondroitin 4-sulfate, low  
iduronate content) of approximately 65 kDa, and a core \*\*\*protein\*\*\*  
after chondroitinase ABC digestion of approximately 45 kDa which also  
contained one to three N-linked oligosaccharides and one O-linked  
oligosaccharide. The other proteoglycan had an overall size of  
approximately 100 kDa, two to three keratan sulfate chains of  
approximately 15 kDa each, and a core \*\*\*protein\*\*\* following  
the elution gradient.

N-linked but no O-linked oligo[**charides**. A larger size, a greater overall hydrophobicity (as measured by its interaction with octyl-Sepharose) and an absence of O-linked oligosaccharides argue that this core \*\*\*protein\*\*\* is a distinct gene product from the core \*\*\*protein\*\*\* of the dermatan sulfate proteoglycan.

2 ANSWER 25 OF 37 MEDLINE DUPLICATE 19  
SESSION NUMBER: 88100442 MEDLINE  
DOCUMENT NUMBER: 88100442 PubMed ID: 3501173  
TITLE: Partial purification and characterization of extrinsic pathway inhibitor (the factor Xa-dependent plasma inhibitor of factor VIIa/tissue factor).  
THOR: Warn-Cramer B J; Maki S L; Zivelin A; Rapaport S I  
REPORTOR SOURCE: Department of Medicine, University of California, San Diego Medical Center 92103.  
TRACT NUMBER: HL 07107 (NHLBI)  
URCE: HL 27234 (NHLBI)  
B. COUNTRY: United States  
CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 198802  
TRY DATE: Entered STN: 19900305  
Last Updated on STN: 20000303  
Entered Medline: 19880202

We report a procedure to \*\*\*purify\*\*\* partially from plasma (approximately 1200 fold) the factor Xa-dependent inhibitor of \*\*\*factor\*\*\* \*\*\*VIIa\*\*\* /tissue factor (i.e., the extrinsic pathway inhibitor or EPI) and describe some of its properties. An assay for EPI was developed based upon inhibition of \*\*\*factor\*\*\* \*\*\*VIIa\*\*\* /tissue factor induced release of activation \*\*\*peptide\*\*\* from tritiated factor IX by a test sample in the presence but not in the absence of factor Xa. Approximately 50% of the total EPI activity in plasma was found in the lipoprotein fraction, which was used as the starting material for \*\*\*purification\*\*\*. Total lipoproteins ( \*\*\*isolated\*\*\* by density ultracentrifugation) were delipidated and the \*\*\*urea\*\*\* soluble apoproteins gel filtered on Sephadryl S-200. The inhibitory activity co- \*\*\*eluted\*\*\* with the major \*\*\*protein\*\*\* peak, which primarily contained apoprotein A-I. Inhibitory activity was separated from apoprotein A-I by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* on Q-Sepharose and was further resolved from higher and lower molecular weight contaminating \*\*\*proteins\*\*\* by polypreparative disc gel electrophoresis in the presence of 0.1% SDS. Functional inhibitory activity \*\*\*eluted\*\*\* from the polypreparative disc gel in two discrete pools of different molecular weights (approximately 34,000 and approximately 43,000 D). Apoprotein E was identified by immunological techniques as the major \*\*\*protein\*\*\* present in both of these pools. However, incubation with a monospecific polyclonal antibody to human apoprotein E did not decrease EPI activity either in plasma or in the partially \*\*\*purified\*\*\* polypreparative disc gel fractions. A rabbit antiserum was prepared against material from the polypreparative disc gel. The IgG fraction neutralized approximately 95% of the total inhibitory activity present in plasma. Therefore, EPI in the lipoprotein fraction and in the non-lipoprotein fraction of plasma appears to be antigenically similar.

12 ANSWER 26 OF 37 CAPLUS COPYRIGHT 2003 ACS  
SESSION NUMBER: 1993:401756 CAPLUS  
DOCUMENT NUMBER: 119:1756  
TITLE: Chromatographic purification of erythropoietin  
INVENTOR(S): Por-Hsiung, Lai; Strickland, Thomas Wayne  
PATENT ASSIGNEE(S): Kirin-Amgen, Inc., USA  
SOURCE: PCT Int. Appl., 20 pp.  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

|   |    |          |                 |          |
|---|----|----------|-----------------|----------|
| WO 8607594                                    | A1 | 1986123  | WO 1986-US1342  | 19860620 |
| W: AU, DK, JP                                 |    |          |                 |          |
| RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE    |    |          |                 |          |
| US 4667016                                    | A  | 19870519 | US 1985-747119  | 19850620 |
| IL 79176                                      | A1 | 19920621 | IL 1986-79176   | 19860420 |
| CA 1297635                                    | A1 | 19920317 | CA 1986-511855  | 19860618 |
| ZA 8604573                                    | A  | 19870225 | ZA 1986-4573    | 19860619 |
| ES 556257                                     | A1 | 19880101 | ES 1986-556257  | 19860619 |
| EP 228452                                     | A1 | 19870715 | EP 1986-904556  | 19860620 |
| EP 228452                                     | B1 | 19950322 |                 |          |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE |    |          |                 |          |
| JP 63503352                                   | T2 | 19881208 | JP 1986-503570  | 19860620 |
| JP 06098019                                   | B4 | 19941207 |                 |          |
| AU 606578                                     | B2 | 19910214 | AU 1986-61230   | 19860620 |
| AU 8661230                                    | A1 | 19870113 |                 |          |
| IL 97135                                      | A1 | 19920621 | IL 1986-97135   | 19860620 |
| AT 120208                                     | E  | 19950415 | AT 1986-904556  | 19860620 |
| DK 8700813                                    | A  | 19870218 | DK 1987-813     | 19870218 |
| CA 1312994                                    | A2 | 19930119 | CA 1991-616009  | 19910221 |
| ORITY APPLN. INFO.:                           |    |          | US 1985-747119  | 19850620 |
|   |    |          | US 1986-872152  | 19860613 |
|   |    |          | CA 1986-5118557 | 19860618 |
|   |    |          | IL 1986-79176   | 19860620 |
|   |    |          | WO 1986-US1342  | 19860620 |

Methods for chromatog. \*\*\*purifn\*\*\* . of erythropoietin from a variety of sources, including biol. fluids or transgenic animal cell lines, is described. The first method is a reversed-phase chromatog. that involves adsorption of the erythropoietin onto a C4 or C6 resin followed by

\*\*\*elution\*\*\* with increasing concns. of EtOH (either stepwise or in a gradient); after removal of EtOH, an erythropoietin fraction of high specific activity with yield >90% is obtained. A second method using \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* on DEAE-agarose at acid pH under conditions that prevent activation of acid proteinases is also described. The two methods may be combined for rapid

\*\*\*purifn\*\*\* . of erythropoietin in high yield and purity. Culture supernatants from CHO cells stably expressing the erythropoietin gene on the plasmid pDSVL-gHuEPO were concd. by diafiltration and fractionated by chromatog. on VYDAC 214TP-B using a 0-80% EtOH gradient in 10 mM tris pH 7.0. The peak of UV absorption \*\*\*eluting\*\*\* around 60% EtOH was pooled and applied to a DEAE-agarose column which was washed with an acid 6M \*\*\*urea\*\*\* buffer to remove proteinases and the \*\*\*urea\*\*\* removed and the column brought to neutral pH with a low-salt buffer. CuSO<sub>4</sub> is optionally present in the wash to assist in oxidn. of sulphydryl groups of undesired \*\*\*protein\*\*\* . Erythropoietin was \*\*\*eluted\*\*\* with a buffer contg. NaCl 75 mM. Final purity of the erythropoietin is >95% and is low in pyrogens and serum \*\*\*proteins\*\*\* .

2 ANSWER 27 OF 37 CAPLUS COPYRIGHT 2003 ACS

CESSION NUMBER: 1985:537917 CAPLUS  
 DOCUMENT NUMBER: 103:137917  
 TITLE: Purification of a neurotrophic factor for ciliary neurons from chick intraocular tissue using nondenaturing conditions  
 AUTHOR(S): Watters, Diane; Hendry, Ian  
 CORPORATE SOURCE: John Curtin Sch. Med. Res., Aust. Natl. Univ., Canberra, Australia  
 SOURCE: Biochemistry International (1985), 11(2), 245-53  
 CODEN: BIINDF; ISSN: 0158-5231  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

3 A neurotrophic factor for the parasympathetic ciliary ganglion neurons was \*\*\*purified\*\*\* to apparent homogeneity from chicken intraocular tissues by high-performance \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . under nondenaturing conditions. The initial stages of \*\*\*purifn\*\*\* . were carried out as described by G. Barbin et al. (1984) up to and including chromatog. on DEAE-cellulose. The 0.25M NaCl eluate from the DEAE-cellulose column was concd. by ultrafiltration, dild., reconcd., and subjected to fast- \*\*\*protein\*\*\* liq. chromatog. on a Pharmacia MonoQ column with gradient \*\*\*elution\*\*\* . The active fractions were pooled, concd., radiolabeled with <sup>125</sup>I, and subjected to electrophoresis on SDS-

of approx. 43,000 daltons and [REDACTED] is not appear to be related to neurotrophic factor \*\*\*isolated\*\*\* from the same source by parative SDS-gel electrophoresis. The final sp. activity of the \*\*\*isolated\*\*\* neurotrophic factor was estd. to be .apprx.4 .times. 105 units/mg.

2 ANSWER 28 OF 37 MEDLINE DUPLICATE 20  
CESSION NUMBER: 86026398 MEDLINE  
CUMENT NUMBER: 86026398 PubMed ID: 3931690  
TITLE: Simultaneous purification of multiple forms of rat liver microsomal cytochrome P-450 by high-performance liquid chromatography.  
THOR: Funae Y; Imaoka S  
URCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1985 Oct 17) 842 (2-3)  
119-32.  
Journal code: 0217513. ISSN: 0006-3002.  
B. COUNTRY: Netherlands  
CUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
NGUAGE: English  
LE SEGMENT: Priority Journals  
TRY MONTH: 198511  
TRY DATE: Entered STN: 19900321  
Last Updated on STN: 19970203  
Entered Medline: 19851127

14 microsomal cytochromes P-450 were \*\*\*purified\*\*\* from the liver of untreated and phenobarbital- or 3-methylcholanthrene-treated male rats. Following solubilization of microsomes with sodium cholate, poly(ethylene \*\*\*glycol\*\*\* ) fractionation and aminohexyl-Sepharose 4B chromatography, cytochromes P-450 were \*\*\*purified\*\*\* by high-performance liquid chromatography (HPLC), using a preparative DEAE- \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*column\*\*\* . The pass-through fraction was further \*\*\*purified\*\*\* by HPLC using a cation-exchange column. Other fractions \*\*\*eluted\*\*\* on preparative DEAE-HPLC were further applied onto an HPLC using a DEAE-column. Five kinds (P-450UT-2-6), four kinds (P-450PB-1,2,4 and 5) and five kinds (P-450MC-1-5) of cytochromes P-450 were \*\*\*purified\*\*\* from untreated rats or rats treated with phenobarbital or 3-methylcholanthrene, respectively. HPLC profiles of tryptic \*\*\*peptides\*\*\* of cytochromes P-450UT-2 and P-450MC-2 were identical and the other profiles obtained from seven \*\*\*purified\*\*\* cytochromes P-450 were distinct from each other. Amino-terminal sequences of eight forms of cytochrome P-450 (UT-2, UT-5, PB-1, PB-2, PB-4, PB-5, MC-1 and MC-5) were distinct except for cytochromes P-450PB-4 and P-450PB-5.

12 ANSWER 29 OF 37 CAPLUS COPYRIGHT 2003 ACS  
CESSION NUMBER: 1984:171197 CAPLUS  
DOCUMENT NUMBER: 100:171197  
TITLE: Partially purified osteogenic factor from demineralized bone  
INVENTOR(S): Seyedin, Saeid; Thomas, Thomas  
PATENT ASSIGNEE(S): Collagen Corp., USA  
OURCE: U.S., 7 pp.  
DOCUMENT TYPE: Patent  
NGUAGE: English  
AMILY ACC. NUM. COUNT: 1  
ATENT INFORMATION:

| PATENT NO.                                    | KIND | DATE     | APPLICATION NO. | DATE     |
|---|------|----------|-----------------|----------|
| US 4434094                                    | A    | 19840228 | US 1983-484286  | 19830412 |
| EP 121976                                     | A2   | 19841017 | EP 1984-300322  | 19840119 |
| EP 121976                                     | A3   | 19860723 |                 |          |
| R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE |      |          |                 |          |
| CA 1223199                                    | A1   | 19870623 | CA 1984-446151  | 19840126 |
| JP 59190919                                   | A2   | 19841029 | JP 1984-14983   | 19840130 |
| JP 63016364                                   | B4   | 19880408 |                 |          |

PRIORITY APPLN. INFO.: US 1983-484286 19830412  
B A process for prepn. of partially \*\*\*purified\*\*\* osteogenic factor from demineralized mammalian, e.g., cattle, pig, bone particles is described. The nonfibrous \*\*\*proteins\*\*\* were extd. from the demineralized bone with a dissociative extractant, e.g., 8M \*\*\*urea\*\*\* or 4M \*\*\*guanidine\*\*\* -HCl, in the presence of a protease inhibitor, N-ethylmaleimide and N-ethylmaleimide. The extd.

nonfibrous \*\*\*proteins\*\*\* were fractionated by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . on DEAE-cellulose at pH 7.0 and the fraction not adsorbed by the DEAE-cellulose was further fractionated by cation-exchange chromatog. on CM-cellulose at pH 4.8. The fraction adsorbed by CM-cellulose was \*\*\*eluted\*\*\* with a NaCl gradient, and the partially \*\*\*purified\*\*\* osteogenic factor was sepd. by gel chromatog. on Sephadex S 200 as a \*\*\*protein\*\*\* with mol. wt. 110,000 daltons. The osteoinductive activity of this \*\*\*protein\*\*\* was assayed by measuring its ability to stimulate rat muscle fibroblasts in agarose to synthesize type II collagen and cartilage proteoglycans.

2 ANSWER 30 OF 37 MEDLINE DUPLICATE 21  
CCESSION NUMBER: 84203512 MEDLINE  
DOCUMENT NUMBER: 84203512 PubMed ID: 6232947  
TITLE: Adenosinetriphosphatase site stoichiometry in sarcoplasmic reticulum vesicles and purified enzyme.  
AUTHOR: Barrabin H; Scifano H M; Inesi G  
CONTRACT NUMBER: HL 27867 (NHLBI)  
SOURCE: BIOCHEMISTRY, (1984 Mar 27) 23 (7) 1542-8.  
Journal code: 0370623. ISSN: 0006-2960.  
SUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198406  
ENTRY DATE: Entered STN: 19900319  
Last Updated on STN: 19970203  
Entered Medline: 19840622

3 The stoichiometry of phosphorylation (catalytic) sites in sarcoplasmic reticulum vesicles ( SRV ) and SR ATPase \*\*\*purified\*\*\* by differential solubilization with deoxycholate was found to be 4.77 +/- 0.4 and 6.05 +/- 0.18 nmol/mg of \*\*\*protein\*\*\* , respectively, when phosphorylation was carried out under conditions permitting <sup>32</sup>P labeling of nearly all sites. Assuming that each site corresponds to a single 115K ATPase chain, the observed site stoichiometry accounts only for 55% and 70% of the total \*\*\*protein\*\*\* . Failure to obtain higher phosphorylation levels was due to the presence of nonspecific \*\*\*protein\*\*\* contaminants in SRV or to the presence of inactive aggregates in the ATPase \*\*\*purified\*\*\* with deoxycholate. This was demonstrated by dissolving SRV and \*\*\*purified\*\*\* ATPase with lithium dodecyl sulfate, subjecting them to molecular sieve HPLC, and collecting the \*\*\*elution\*\*\* fractions for determination of \*\*\*protein\*\*\* , measurement of <sup>32</sup>P-labeled sites, and electrophoretic analysis. In fact, in the specific \*\*\*elution\*\*\* peak containing the 115K ATPase chains, phosphorylation levels were 6.62 +/- 0.33 and 7.03 +/- 0.18 in SRV and \*\*\*purified\*\*\* ATPase, corresponding to 68% and 86% of the \*\*\*protein\*\*\* in the specific \*\*\*elution\*\*\* peak. An alternate \*\*\*purification\*\*\* method was then developed, based on solubilization of SRV with dodecyl octaethylene \*\*\*glycol\*\*\* monoether ( C12E8 ), separation of delipidated ATPase by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* , and enzyme reactivation with phosphatidylcholine. This preparation yields 7.3 +/- 0.44 nmol of phosphorylation site/mg of \*\*\*protein\*\*\* of the SRV fraction before HPLC. (ABSTRACT TRUNCATED AT 250 WORDS)

12 ANSWER 31 OF 37 MEDLINE DUPLICATE 22  
CCESSION NUMBER: 84202839 MEDLINE  
DOCUMENT NUMBER: 84202839 PubMed ID: 6721830  
TITLE: Studies on the alpha-subunit of bovine brain S-100 protein.  
AUTHOR: Masure H R; Head J F; Tice H M  
SOURCE: BIOCHEMICAL JOURNAL, (1984 Mar 15) 218 (3) 691-6.  
Journal code: 2984726R. ISSN: 0264-6021.  
SUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198406  
ENTRY DATE: Entered STN: 19900319  
Last Updated on STN: 19900319  
Entered Medline: 19840608

\*\*\*protein\*\*\* and calmodulin from crude bovine brain extracts by the use of a fluphenazine-Sepharose affinity column \*\*\*eluted\*\*\* likewise with decreasing concentrations of free Ca<sup>2+</sup>. \*\*\*protein\*\*\* containing only alpha-subunit was \*\*\*purified\*\*\* from preparations of S-100 \*\*\*protein\*\*\* by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatography\*\*\* . This \*\*\*protein\*\*\* co-migrated with the alpha-subunit of S-100 \*\*\*protein\*\*\* on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and had an amino acid composition identical with that previously reported for this subunit. The results of u.v.-absorption and fluorescence-emission spectroscopy indicate that the tryptophan residue of the \*\*\*purified\*\*\* alpha-subunit of S-100 \*\*\*protein\*\*\* undergoes a Ca<sup>2+</sup>-induced change in environment. Measurements of changes in tryptophan fluorescence with increasing Ca<sup>2+</sup> concentrations suggest an apparent dissociation constant of the alpha-subunit for Ca<sup>2+</sup> of  $7 \times 10^{-5}$ M in the absence of K<sup>+</sup>. In the presence of 90mM-K<sup>+</sup> this value is increased to  $3.4 \times 10^{-4}$ M.

ANSWER 32 OF 37 CAPLUS COPYRIGHT 2003 ACS

SESSION NUMBER: 1984:47927 CAPLUS  
CUMENT NUMBER: 100:47927  
TITLE: Ion-exchange chromatography of proteins using diethylaminoethyl-Sephadex in the presence of urea  
THOR(S): Kadoya, Toshihiko; Okuyama, Tsuneo  
REPORT SOURCE: Fac. Sci., Tokyo Metrop. Univ., Tokyo, Japan  
URCE: Bunseki Kagaku (1983), 32(11), 664-9  
CUMENT TYPE: Journal  
NGUAGE: Japanese  
To \*\*\*purify\*\*\* cytoskeletal \*\*\*proteins\*\*\* such as glial fibrillar acidic \*\*\*protein\*\*\* (GFA) or neurofilament \*\*\*proteins\*\*\* (NFP), the \*\*\*proteins\*\*\* were extd. with 2M \*\*\*urea\*\*\* from brain tissue. \*\*\*Anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . was done on a 6 times. 0.75 cm (inner diam.)-column packed with DEAE-Sephadex A 50 equilibrated in 10 mM K phosphate buffer (pH 7.1) with or without \*\*\*urea\*\*\* , and a linear NaCl gradient was used for \*\*\*elution\*\*\* of \*\*\*proteins\*\*\* . Human IgG, bovine serum albumin (BSA), and human serum \*\*\*proteins\*\*\* were used as stds. In the presence of \*\*\*urea\*\*\* , interaction between the ion-exchanger and BSA was reduced. In the presence of 6M \*\*\*urea\*\*\* , the \*\*\*elution\*\*\* patterns of the \*\*\*proteins\*\*\* were affected; however, 2M \*\*\*urea\*\*\* scarcely produced an effect on ion-exchange chromatog. of human serum \*\*\*proteins\*\*\* . A 2M \*\*\*urea\*\*\* ext. of bovine brain, which was rich in GFA and NFP, was sepd. on DEAE-Sephadex A 50 in the presence of 2M \*\*\*urea\*\*\* .

2 ANSWER 33 OF 37 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 23

SESSION NUMBER: 1982:595277 CAPLUS  
CUMENT NUMBER: 97:195277  
TITLE: Purification of the glial fibrillary acidic protein by anion-exchange chromatography  
THOR(S): Dahl, Doris; Crosby, Carol J.; Gardner, Eileen E.; Bignami, Amico  
REPORT SOURCE: Spinal Cord Injury Res. Lab., West Roxbury Veterans Adm. Med. Cent., Boston, MA, 02132, USA  
URCE: Analytical Biochemistry (1982), 126(1), 165-9  
CUMENT TYPE: Journal  
NGUAGE: English

A procedure for the \*\*\*isolation\*\*\* of assembly-competent glial fibrillary acidic (GFA) \*\*\*protein\*\*\* from 2M \*\*\*urea\*\*\* exts. of bovine spinal cord by \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . is reported. The tissue was previously extd. with low-ionic-strength buffer. The procedure allowed the sepn. of nondegraded GFA \*\*\*protein\*\*\* from GFA \*\*\*protein\*\*\* comprising degraded species. As previously reported for neurofilament preps. obtained from porcine spinal cord by N. Geisler and K. Weber (1981), the procedure also allowed the simultaneous sepn. of the 3 neurofilament \*\*\*polypeptides\*\*\* (200,000; 150,000; and 70,000 daltons) contained in the 2M \*\*\*urea\*\*\* ext. Brain filament \*\*\*proteins\*\*\* sequentially \*\*\*eluted\*\*\* at increasing salt concn. (25-200 mM NaCl) according to their isoelec. point. \*\*\*Proteins\*\*\* with higher pI \*\*\*eluted\*\*\* first. Tubulin

\*\*\*polypeptides\*\*\* .

ANSWER 34 OF 37 CAPLUS COPYRIGHT 2003 ACS

SESSION NUMBER: 1980:617475 CAPLUS

JUMENT NUMBER: 93:217475

TLE: Radioligand assays - methods and applications. V.  
125I-monoiodoinsulin - preparation, immunological and  
biological characterization

THOR(S): Besch, W.; Woltanski, K. P.; Knospe, S.; Ziegler, M.;  
Keilacker, H.

RPORTATE SOURCE: Abt. Radioimmunol., Zentralinst. Diabetes "Gerhardt  
Katsch", Karlsburg, 2201, Ger. Dem. Rep.

JRCE: Acta Biologica et Medica Germanica (1980), 39(4),  
495-502

CODEN: ABMGAJ; ISSN: 0001-5318

CUMENT TYPE: Journal

NGUAGE: German

Monoiodoinsulin was prep'd. from a heterogeneous 125I-iodination mixt. by  
\*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* on DEAE-Sephadex A  
25 without gradient \*\*\*elution\*\*\* (40 mM Tris, 0.1M NaCl, and 7M  
\*\*\*urea\*\*\* at pH 9.0 and 4.degree.). The sp. radioactivity of  
[125I]monoiodoinsulin was 14.3 TBq/g, i.e., an I content of 1.04  
atoms/mol. \*\*\*insulin\*\*\*. Monoiodoinsulin was indistinguishable from  
native \*\*\*insulin\*\*\* with respect to binding to guinea pig antiinsulin  
serum and to \*\*\*insulin\*\*\* \*\*\*receptors\*\*\* of \*\*\*isolated\*\*\*  
rat adipocytes. The biol. potency (96.5% of the immunoreactive  
\*\*\*insulin\*\*\* activity) detd. by the conversion of [1-14C]D-glucose to  
14CO2 in vitro by rat fat cells was similar to that of native  
\*\*\*insulin\*\*\*.

2 ANSWER 35 OF 37 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE

24

SESSION NUMBER: 1978:214900 BIOSIS

CUMENT NUMBER: BA66:27397

TLE: HETEROGENEITY POLY PEPTIDE CHAIN COMPOSITION AND ANTIGENIC  
REACTIVITY OF COMPLEMENT C-3 NEPHRITIC FACTOR.

THOR(S): DAHA M R; AUSTEN K F; FEARON D T

RPORTATE SOURCE: DEP. MED., LEIDEN UNIV., LEIDEN, NETH.

URCE: J IMMUNOL, (1978) 120 (4), 1389-1394.

CODEN: JOIMA3. ISSN: 0022-1767.

LE SEGMENT: BA; OLD

NGUAGE: English

C3 [3rd component of complement] nephritic factor (C3NeF), recognized by  
its capacity to stabilize the cell-bound amplification C3 convertase,  
C3b,Bb, was \*\*\*purified\*\*\* from sera of 3 patients with  
hypocomplementemic glomerulonephritis and of 2 patients with partial  
lipodystrophy by QAE-A50 Sephadex and SP C-25 Sephadex chromatography,  
affinity for the fluid phase amplification C3 convertase and QAE-A50  
Sephadex chromatography. Each C3NeF preparation exhibited heterogeneity  
during cation exchange chromatography. The isoelectric points of the

\*\*\*eluted\*\*\* fractions ranged at pI 8.3-8.9. The chromatographic  
fractions were interacted with \*\*\*purified\*\*\* B, .hivin.D and C3 to  
form fluid phase C3b,Bb(C3NeF) which sedimented as a 10S complex on  
sucrose density gradient ultracentrifugation; the \*\*\*isolated\*\*\*  
convertase was decayed with release of C3NeF, which was separated from C3b  
and Bi [inactivated Factor B] by \*\*\*anion\*\*\* \*\*\*exchange\*\*\*

\*\*\*chromatography\*\*\*. \*\*\*Purified\*\*\* preparations of C3NeF  
radiolabeled with 125I were bound from 92-98% by 109 erythrocytes bearing  
C3b,Bb. Erythrocytes carrying C3b bound from 0.6-18% and EA  
[antibody-sensitized erythrocytes] engaged in no specific uptake. Analysis  
of all 125I-C3NeF preparations by SDS-PAGE [sodium dodecyl  
sulfate-polyacrylamide gel electrophoresis] demonstrated an apparent MW of  
150,000. After reduction in the presence of 8M \*\*\*urea\*\*\*, each  
125I-C3NeF preparation revealed \*\*\*polypeptide\*\*\* chains of 54,000 and  
23,500 MW which corresponded with the positions of the H and L chains of  
reduced Ig[immunoglobulin]G. The reaction of 125I-C3NeF from 4 patients  
was positive with Sepharose-bound antisera to IgG, .alpha.1, .alpha.2,  
.kappa. and .lambda. and negative with antisera to .mu., .alpha., .delta.,  
.gamma.3 and .gamma.4. C3NeF from the 5th patient differed in not reacting  
with antiserum to .kappa.. C3NeF is probably an autoantibody directed  
against the antigens expressed by the amplification C3 convertase, C3b,Bb.

12 ANSWER 36 OF 37 CAPLUS COPYRIGHT 2003 ACS  
CCESSION NUMBER: 1976:16132 CAPLUS  
DOCUMENT NUMBER: 84:161321  
TITLE: Purification of factor XII (Hageman factor) from human plasma  
UTHOR(S): Chan, John Y. C.; Movat, Henry Z.  
CORPORATE SOURCE: Inst. Immunol., Univ. Toronto, Toronto, ON, Can.  
OURCE: Thrombosis Research (1976), 8(3), 337-49  
OCUMENT TYPE: Journal  
ANGUAGE: English

B Blood-coagulation factor XII was \*\*\*purified\*\*\* from human plasma in 4 steps in the presence of hexadimethrine bromide and soybean trypsin inhibitor (SBTI). After adsorption with aluminum hydroxide, the plasma was pptd. with polyethylene \*\*\*glycol\*\*\* . The \*\*\*protein\*\*\* precipitating between 4.0 and 16% satn. was redissolved in .apprx.30% of the starting plasma vol. and chromatographed. The 1st chromatographic step was \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . on QAE-Sephadex, in the presence of hexadimethrine bromide and SBTI. The SBTI was used also during the 1st half of chromatog. on CM-Sephadex, from which factor XII \*\*\*eluted\*\*\* in the 2nd half. As a 4th preparative step factor XII was subjected to either gel filtration on Sephadex G 100 or affinity chromatog. The latter consisted of an immunoabsorbent column, the antibody being against contaminating \*\*\*proteins\*\*\* \*\*\*isolated\*\*\* from factor XII-deficient plasma. The final product exhibited a sharp intense band by Na dodecyl sulfate disc gel electrophoresis, with an estd. mol. wt. of 78,000.

12 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2003 ACS  
CCESSION NUMBER: 1971:135269 CAPLUS  
DOCUMENT NUMBER: 74:135269  
TITLE: Urate-binding alpha1-2 globulin. Isolation and characterization of the protein from human plasma  
UTHOR(S): Aakesson, I.; Alvsaker, J. O.  
CORPORATE SOURCE: Dep. Biochem., Univ. Oslo, Bindern, Norway  
OURCE: European Journal of Clinical Investigation (1971), 1(4), 281-7  
OCUMENT TYPE: Journal  
ANGUAGE: English

B The urate-binding alpha1-2-globulin occurred mainly in the human plasma \*\*\*protein\*\*\* fraction \*\*\*eluted\*\*\* from DEAE-Sephadex columns with 0.01M Na phosphate buffer, pH 7.35, contg. 0.40M NaCl. By \*\*\*anion\*\*\* - \*\*\*exchange\*\*\* \*\*\*chromatog\*\*\* . of the fraction on DEAE-Sephadex columns, followed by (NH4)2SO4 pptn. and preparative polyacrylamide gel electrophoresis, this urate-binding globulin was \*\*\*isolated\*\*\* in a highly \*\*\*purified\*\*\* state. It was a rod-shaped glycoprotein with a mol. wt. of 67,000 as detd. by dodecyl sulfate-polyacrylamide gel electrophoresis and had an isoelec. point of pH 4.6. In the presence of 6M \*\*\*urea\*\*\* and mercaptoethanol the \*\*\*protein\*\*\* did not split into subunits, indicating that it might represent a single \*\*\*polypeptide\*\*\* chain. The urate-binding globulin contained 12.1% carbohydrate, including galactose, mannose, galactosamine, and sialic acid. Its amino acid compn. did not differ significantly from that of other plasma \*\*\*proteins\*\*\* except for the presence of an unidentified compn. The urate-binding globulin did not seem to be identical to any previously characterized \*\*\*protein\*\*\* from human blood.

> d his

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1 10512953 S PEPTIDE OR POLYPEPTIDE OR OLIGOPEPTIDE OR PROTEIN OR RECEPTOR  
2 13965 S (FACTOR VIIA) OR (FACTOR VIIIAI) OR (FFR-FACTOR VIIA) OR (GLUC  
3 1457073 S (L1 OR L2) (P) (PURIF? OR ISOLAT?)  
4 30144 S ANION (W) EXCHANGE (W) (CHROMATOGRAPHY OR COLUMN)  
5 8956 S L3 (P) L4  
6 5826 S ORGANIC MODIFIER  
7 890678 S ALKANOL OR ALKYNOL OR ALKENOL OR UREA OR GUANIDINE OR (ALKNAO

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0 S L9 (P) IMPURIT?  
103 S L9 (P) ELUT?  
37 DUPLICATE REMOVE L11 (66 DUPLICATES REMOVED)  
0 S L9 (P) (NEGATIVE CHARGE)

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